Essential oils against bacterial biofilm formation and quorum sensing of food-borne pathogens and spoilage microorganisms

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Bacterial attachment and biofilm formation cause severe hygiene problems in the food industry. Biofilms can be formed on different equipment surfaces (stainless steel, plastic, rubber, teflon, glass) and also on foods (meat, dairy and seafood products, vegetables, fruits, brewing). Extracellular polymeric substances are the protecting material of these structures and represent the barrier between the environment and the bacteria making them less sensitive to disinfectants. These structures can be formed by bacteria and yeast as well, but the most common forms in nature are the mixed-culture biofilms. Most of these biofilms harbor also pathogens therefore biofilms are a continuous source of contamination that may lead not only to food spoilage but also transmission of diseases. Raw products like poultry meat, vegetables, fruits, sea food have frequently been found to be contaminated with pathogenic microorganisms like *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli*. When talking about biofilms the density-dependent cell-to-cell communication called quorum sensing (QS) has to be mentioned. QS plays an important role in biofilm development, resistance and virulence. QS signaling molecules can be oligopeptides in Gram-positive bacteria, N-acyl homoserine lactones (AHLs) in Gram-negative bacteria and autoinducer-2 (AI-2) in both Gram-negative and Gram-positive bacteria. Controlling this mechanism could be a potential strategy against the development of resistance. The conventional control sanitizing strategies are chemicals (chlorine, hydrogen peroxide, ozone, peracetic acid) and ultrasonication, enzymes, phages, preservatives, heat treatment or refrigeration. The number of usable chemicals in food and eco-food industry is limited and their use can often produce unpleasant by-products. Nowadays customers prefer products that are minimally processed and have fewer chemicals so it has become necessary to find natural and effective cleaning and preserving products. There is a growing interest in using essential oils (EOs) as natural preservatives and sanitizers in the food industry. EOs damage the cell wall and membranes of microorganisms, alter the morphology and coagulate the cytoplasmatic material. Most of them are recognized as safe (GRAS) and can be used directly in foodstuff. Latest research and our experiments reported the good antimicrobial effects of EOs on pathogenic and spoilage bacteria. Besides this, they have good anti-biofilm forming and anti-QS effect. The strong aroma of EOs can affect organoleptic properties of foods. Our results showed that it is possible to find a dose of EOs which combines antimicrobial efficiency with a pleasant flavour effect. These results lead to the conclusion that EOs can be used as alternative sanitizers and preservatives in the food industry.

Keywords: biofilm; essential oils; food spoilage bacteria; food-borne pathogens; quorum sensing

1. Biofilms and their occurrence

1.1 Bacterial biofilms

A biofilm is a matrix of microorganisms with extracellular substances (EPS) that can be formed on different surfaces. These surfaces can be animal tissue (meat, fish products), catheter, teeth, stainless steel, plastic, glass, teflon, rubber, wood, etc. Biofilm formation can be divided into 5 parts. First (1-2): reversible and irreversible attachment to the surface; 3: development of the extracellular matrix; 4: maturation of the biofilm and; 5: dispersion. In reversible stage, the bacteria attach to surfaces with van der Waals attraction forces, electrostatic forces and hydrophobic interactions. During this phase, bacteria can be removed from surfaces easily for example by rinsing. If bacteria interact with surfaces by dipole-dipole, hydrogen, ionic or covalent bonding, removal will be more difficult [1, 2]. The finishes of stainless steel surfaces also influence bacterial attachment. The adherence is stronger to untreated or sandblasted surfaces than to electropolished area [3]. After attachment the bacteria form microcolonies and produce a matrix called extracellular polymeric substance (EPS). The EPS protects bacteria within the biofilm (cells are more tolerant to stress factors) and is responsible for binding. It is composed of polysaccharides, proteins, nucleic acids, lipids [4]. When biofilms consists of different microorganisms, the matrix is thicker and more stable than the matrix of single species biofilms [1]. If the biofilm reaches the maximum, starvation, enzymatic degradation and increased fluid shear will appear. The cells from the top of the biofilm will disperse and colonize new surfaces [2]. Biofilms can occur in different industrial and medical environments. 65-80% of infections are related to biofilms [5]. In the food industry biofilms can be found in dairy, fish processing, poultry meat and ready-to-eat food factories and can cause serious hygiene and technological problems by cross contamination [2]. Biofilms have part in outbreaks of pathogens and increase the risk of contamination in food plants. Bacterial matrix forming ability of *Pseudomonas, Acinetobacter, Moraxella,*

Brochothrix thermosphacta, Lactobacillus, Enterococcus was reported in detail [6]. *Salmonella* species also form matrix on many types of surfaces and equipments in food production [7]. *Pseudomonas spp*. produce enzymes, some of those can survive the ultra-high-temperature and reduce the shelf-life of foods. *Bacillus cereus, Escherichia coli*, *Shigella* sp. and *Staphylococcus aureus* were isolated from dairy processing line [8]. *Pseudomonas* spp. was found in high percentage in fish processing and intensified the colonization of *L. monocytogenes* [9]. *L monocytogenes* was found on filling or packaging equipments, slicers, freezers, conveyors and containers. It can be transferred from walls, equipment, coolers, and floors [10]. This bacterium is able to multiply at few degrees above 0 °C (in refrigerated premises), and growing and multiplying *L. monocytogenes* was found on floors, drains, cold and wet atmosphere rooms. [11]. Recently, *E. coli* biofilms were isolated from beef-contact surfaces [2].

1.2 Cell-to-cell communication (quorum sensing)

Quorum sensing (QS) is a population-dependent phenomenon based on signal molecules first characterized in the 1970s in luminescent marine species of *Vibrio.* Studies show that this phenomenon is widely spread among bacteria [12, 13]. The production of antibiotics, virulence factors of pathogens, exoenzymes and resistance are regulated by QS. The signal molecule production is based on an autoinducing mechanism and the type of the molecules differs between Gram-positive and Gram-negative bacteria. Common classes of signaling molecules are oligopeptides in Gram-positive bacteria, N-acyl homoserine lactones (AHLs) in Gram-negative bacteria and autoinducer-2 (AI-2) in both Gram-negative and Gram-positive bacteria. Quorum sensing also participates in biofilm formation. Studies show that quorum sensing-deficient mutants have been shown to form thinner and more unstructured biofilms compared to the wild types (14, 15). The QS system of *Serratia marcescens* regulates the production of virulence factors and its biofilm formation so it is involved in the pathogenesis of the bacterium [16]. This leads to the conclusion that by inhibiting this process the pathogenesis and biofilm formation could be influences. Resistance of bacteria against antibiotics and sanitizers has become a major problem over the years. Those anti-QS agents that do not exert selective pressure over the bacteria that lead to resistance are of particular interest [16]. Such inhibitors have been discovered recently, some bioactive compounds from marine bacteria, the red alga *Delisea pulchra* and essential oils of plants have shown to be effective against QS [16, 17, 18, 19, 20]. It has recently been proven that QS signal molecules are present on foods and play an important role in food spoilage and that these signal molecules are produced by certain members of the initial microbial association [21]. In order to meet consumers demand regarding avoiding of artificial food preservatives new strategies and compounds are needed. A possibility would be to inhibit QS with natural preservatives that do not have negative influence over the taste and odour of foods.

1.3 Biofilms in the food industry

1.3.1 Biofilms on equipments - classic cleaning and sanitizing methods

For successful inhibition of biofilm formation the first step is the prevention of cell attachment. In the food industry different methods are used for this purpose: frequent sanitizing, appropriate implementation and control of the Good Hygiene Practice (GHP) and Hazard Analysis and Critical Control Points (HACCP), the use of electro polished surfaces, antibacterial and anti-adhesive coatings, and surfaces with increased hydrophobicity [2, 22]. Disinfectants like hydrogen peroxide, quaternary ammonium compounds, chlorine, sodium hypochlorite, peracetic acid (PAA), and EDTA are used during disinfection [1, 23]. These chemicals showed different activity against biofilms; chlorine was less effective on multispecies biofilms (*Pseudomonas* sp. and *L. monocytogene*s) on stainless steel than PAA. *Listeria innocua* was more resistant to sodium hypochlorite and PAA in multispecies biofilms than in single cultures on different surfaces [23]. Shi and Zhu [8] reported that *Pseudomonas aeruginosa* and *Staphylococcus aureus* were resistant to Easyclean (an alkaline detergent) and Ambersan (an acidic cleaner) sanitizers in biofilm form. These chemicals reduced the cell number with only 1 log CFU. *L. monocytogenes* showed also resistance to quaternary ammonium compounds and commercial hydrogen peroxide-based agent could not eliminate the *L. monocytogenes* biofilm. Chemicals used for clean-in-place (CIP) hygiene method were not effective on biofilms, thus the addition of some biological materials (enzymes) was recommended in CIP system. Hydrogen peroxide at small concentration was an effective disinfectant against biofilms [8]. The use of traditional chemical disinfectants raises some problems: they can cause environmental pollution and in the presence of organic materials harmful by-products can be formed. In recent years alternatives were proved for biofilm elimination like enzymes (Endo-H), polysaccharides (for hydrolyzing), bacteriocins (nisin, surfactin) and plant extracts (essential oils) [1, 2]. There are also physical methods for destroying the bacterial matrix: automated scrubbing, pressure-jet washing, ionizing radiation and ultrasound treatment [24]. Despite the efforts have been made biofilm removal remained a great challenge.

1.3.2 Biofilms on food surface – classic preservation methods

The shelf life of foods can be defined as the time period within which the food is safe to consume and/or has an acceptable quality to consumers. Preservation involves reduction of the microbial load or limitation of growth of

microorganisms and shelf-life extension relies on changing storage conditions or packaging to inhibit microbial growth. High-quality ingredients are needed for effective preservation. Common methods of preservation include pickling, drying, adding sugar or salt, smoking, chilling, freezing or vacuum packaging is used [25]. Contamination of foods can occur through contact with processing surfaces, the hand of workers, the water used for washing, the air. The presence of bacterial biofilms on food surfaces has been demonstrated in several occasions. Morar and co-workers [26] demonstrated the presence of clusters of microorganisms included in a biofilm matrix on surface of pig carcasses from sternal, coast and coccigian region and also on cut pieces with epifluorescent microscope. They also demonstrated that the level of contamination depended on the humidity level and the structure of the meat. Higher fat content resulted in a lower CFU [26]. In case of fruits pre- and post-harvesting factors can affect the microbial quality [27]. The processing methods involve the use of temperature, moisture content and ethylene control. Normal microflora of fruits is diverse and includes bacteria such as *Pseudomonas*, *Erwinia*, *Enterobacter*, and *Lactobacillus* sp. and a variety of yeasts and molds [28]. These microbes remain adhered to outer skin of fruits and come from several sources such as air, soil, compost, and insect infestation. Wade and Beuchat have well documented the crucial role of proteolytic fungi and the associated implications on the changes in pH of the pericarp of the decayed and damaged raw fruits in survival and growth of various food-borne pathogens [29]. *Botrytis* or *Rhizopus* spoilage of fruits could help create environment for the proliferation of *Salmonella* Typhimurium [30], while Dingman observed the growth of *E. coli* 0157:H7 in bruised apple tissues [31]. Biofilm formation of *Salmonella spp*. was reported on cantaloupe surface and associated with *Salmonella* outbreaks [32]. Pathogens can survive for relatively long times in water, or in plant residue entrapped in process line equipment or biofilms, and can subsequently contaminate clean product that passes through that water [33]. In this case, common forms of sanitization are halogenated sanitizers (chlorine, bromine, and iodine), ozonation, hydrogen peroxide, quaternary ammonium compounds. The discovery of compounds that inhibit cell-to-cell communication could provide a novel method for fighting against microbes [34]. Because QS regulates attachment and biofilm formation the inhibition of this process with natural agents could be a new possibility for food preservation [16, 35].

2. Essential oils

Essential oils (EOs) are aromatic hydrophobic liquids originated from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, effleurage steam distillation or extraction. As estimated 3000 EOs are known, can comprise more than 60 individual compounds and about 300 EOs are commercially important [36, 37.] Plants produce a variety of compounds with antimicrobial activity [38]. The antifungal activity of EOs is related to their components, the structural configuration of these components and their functional groups and possible synergistic interactions between components [39].

Compounds of EOs are generally classified into 2 groups of distinct biosynthetical origin. The main group contains terpenes and terpenoids whereas the other consists of aromatic compounds (phenylpropanoids). Terpenes are hydrocarbons made up of several isoprene units and terpenes containing oxygen are called terpenoids. Examples of terpenes present in EOs are p-cymene, terpinene, limonene, sabinene, and pinene.

Terpenoids can be subdivided into alcohols, esters, aldehydes, ketones, ethers, and phenols. Geraniol, menthol, linalool, citronellol, carvone, thymol, carvacrol, geranyl acetate, eugenyl acetate, geranial, neral and 1,8-cineole are the well-known terpenoids found in EOs. Cinnamaldehyde, cinnamyl alcohol, chavicol, eugenol, estragole, methyl eugenols and methyl cinnamate are phenylpropanoids [40, 41, 42].

Most components in EOs have several targets. It is therefore difficult to predict how susceptible a microorganism is and why the susceptibility varies from strain to strain. Predictions about the mode of action of crude essential oils require thorough investigations of their constituents' target site, their mode of action, and their interactions with the surrounding environment [43].

The phenolic components are most active and responsible as membrane permeabilisers. Gram-positive organisms are generally more sensitive to EOs than the gram-negative ones [44, 45].

Increased membrane permeability leads to proton, phosphate and potassium leakage, which further affects pH homeostasis and equilibrium of inorganic ions [45]. The lysis may also have been due to weakening of the cell wall and the latter rupture of the cytoplasmic membrane due to osmotic pressure. Nucleic acids are lost through the damaged cytoplasmic membrane [46]. EOS can inhibit the mitochondrial ATPase activity and reduce the mitochondrial membrane potential in cells. They could be effective on the activity of the mitochondrial dehydrogenases of fungi and in inhibition the glucose-induced reduction of external pH in a time- and concentration-dependent manner [47].

There are also some studies about the morphological changes caused by EOs: the apparent rupture of the cell wall and leakage of the cytoplasmic contents could be observed, fungal hyphae appear flattened and wrinkled.

EOs could be effective in inhibiting of mycotoxin and ergosterol production [48]. EO components are able to activate a compensatory mechanism which generates an adaptive response of the fungus resulting in the reprogramming of genomic expression to protect the cell-wall structure [49].

3. Methods for biofilm detection

The main obstacle for using essential oils as antimicrobials and food preservatives is that most often they cause negative organoleptic effects when added in sufficient amounts to provide an antimicrobial effect. This is why it is important to determine minimum inhibitory concentrations and minimal bactericide concentrations before use. The literature presents different methods that can be used.

a. Agar dilution assay

The essential oils are added to cooled medium in different concentration range. The medium is split in Petri-dishes and the inoculum is plated. The plates are incubated at appropriated temperature for 24 h and the MIC is determined as the lowest concentration where the microorganisms do not grow [50].

b. Disc diffusion assay

The cell suspension is plated, filter paper discs (6 mm) are placed on the plate and different amounts of EOs are added to the disc. Petri dishes are incubated at appropriated temperature for the required time. The inhibition zones are measured [51].

c. Resazurin assay

The microorganisms are mixed with different concentrations of EOs in 96 well microtiter plates. After incubation 10 µl resazurin is added to each well and after 2 h the absorbance is measured at 570 nm [52]. The color of resazurin changes from blue to pink, because it is reduced to resorufin by oxidoreductases of viable cells [53].

d. Measuring optical density

After incubation of inoculated microtiter plates the optical density of each well is measured at 595 nm [54]. Absorbence is evaluated taking into account the negative and positive controls (cell free solutions and non-treated solutions).

e. Checkerboard method

Different concentration of EOs are mixed in 1:1 rations and added to the cell suspension. After 24 hours incubation the fractional inhibitory concentration (FIC) is calculated as follows (x, y):

$$FIC\ (x) = \frac{MIC\ (x)\ in\ combination}{MIC\ (x)\ alone}$$

$$FIC\ (y) = \frac{MIC\ (y)\ in\ combination}{MIC\ (y)\ alone}$$

FIC index (FIC(x)+FIC(y)) is used for assessment of interaction: FICI < 0.5 synergy; 0.5≤FICI≤1 additive; 1<FICI<4 indifferent; FICI>4 antagonist [53]. Minimal bactericidal concentration (MBC) is the lowest concentration where bacteria show no growth on agar after plating cell suspensions from MIC determination [55].

The cell number of biofilms can also be quantified. This can be made by swabbing, vortexing, shaking with beads or sonication of surfaces or staining with specific dyes and measuring absorbance. Staining can be conducted with tetrazolium salt (XTT) – Menadione reagent. After incubation the colorimetric change is measured at 450 nm and correlated to positive and negative controls [56, 57]. Crystal violet staining method (CV) is usually used for biofilm elimination detection. Inhibitors and bacterial suspensions are mixed in 1:1 ratio and after incubation at appropriate temperatures washing steps are conducted to remove the planktonic cells. To quantify the amount of biofilm formed different CV is used and after removing the excess dye bound crystal violet is released by adding acetic acid. Finally absorbance is measured [57]. Another method for biofilm detection is microscopy. There are epifluorescent, scanning electron, confocal laser scanning, and atomic force microscopes for biofilm imaging. 0.001% acridine orange solution is used for biofilm staining on epifluorescence microscopy [58]. By fluorescence microscopy some dyes are used like CTC-DAPI, LIVE/DEAD kit BacLight™, SYTO 9, FITC labeled concanavilan A [58, 59].

The main preparation steps for scanning electron microscopy are fixing the samples with glutaraldehyde, drying with different concentration of ethanol and ethanol:buthanol, freeze-drying and coating with gold [20].

4. Anti-biofilm forming effect of EOs

Frequently investigated species regarding their biofilm formation are Staphylococci, Enterobacteriacee and the food-borne pathogen *Listeria monocytogenes.* Several EOs and components have been investigated against these and other pathogens as well. Oils like citrus EOs, clary sage, marjoram, oregano, thyme, cinnamon, and their major components have been shown to be effective against the biofilm forming ability of some of these pathogens. Cassia, Peru balsam and red thyme essential oils proved to be more effective in eradicating Pseudomonas and *S. aureus* biofilms than some important antibiotics [60]. Oregano EO, carvacrol and thymol inhibited biofilm formation of *Staphylococcus aureus* and *Staphylococcus epidermidis* strains but twofold and fourfold greater concentrations were needed than in case of planktonic cells. The essential oil of *Satureja thymbra* as well as its hydrosol fraction exhibited a strong antimicrobial

action against both monospecies and mixed-culture biofilms [61]. Biofilm of isolated bacteria from industrial beverage filling plant (*Sphingomonas, Stenotrophomonas* and *Acinetobacter* spec.) was inhibited with thyme and oregano EO, where the EOs concentration was 0.004%-0.031% [62].

5. Anti-QS effect of EOs

There is an ongoing need for investigating herbal products in the quest for new anti-pathogenic agents that might act as nontoxic inhibitors of QS, thus controlling infections without leading to the appearance of resistant bacterial strains. Anti-QS agents were first characterized in the red marine alga (*Delisea pulchra*) [17] and a few higher plants [63, 64]. The discovery of halogenated furanons produced by the algae *D. pulchra* which interfered with N-acyl homoserine lactone regulatory system of Gram-negative bacteria was an important discovery. Since then several plant extracts have also been tested for their antagonistic effect against QS [18, 65, 66, 67, 68, 69]. Essential oils have shown to be effective anti-QS agents as well [19, 20, 70, 71, 72]. EOs of tea tree, rosemary, ginger, rose, chamomile, eucalyptus, marjoram, clary sage, juniper and several others showed moderate or intensive anti-QS effect [19, 20]. It has been demonstrated that QS regulates biofilm formation of bacteria [73]. Studies also reveal that different EOs have been observed to be effective against biofilms formed by pathogens like *Salmonella*, *Listeria*, *Pseudomonas* and *Staphylococcus* [7, 43, 68, 74, 82]. Several methods have been used to demonstrate the anti-QS effect of EOs. The most common methods are disc and agar diffusion or flask-incubation assays. The violacein producing *Chromobacterium violaceum* is generally used for these investigations because its pigment production is regulated by QS. Usually agar plates inoculated with *C. violaceum* are used and paper discs are impregnated with the investigated EO. If the production of AHL molecules is inhibited than colorless halos will appear around the paper disc indicating that cells survived but their communication has been disrupted [19, 20]. Besides these model bacteria biosensor strains have also been used to aid the screening of compounds with anti-QS abilities. For example *C. violaceum* 026 is a Tn5 mutant strain that produces violacein upon induction with externally added short-chain autoinducers [74]. *Agrobacterium tumefaciens* NTL4 and A136 sense long chained AHL molecules and *Vibrio harvey* BAA-1117 senses AI2 signal molecules [21].

6. Essential oils as sanitizers

Biofilm eliminating effect of EOs is well documented. In most papers biofilms of food pathogens were eliminated with the aid of thyme, oregano, lemongrass and cinnamon EO. De Oliveira [45] reported about the effect of *Cymbopogon citratus* and C. *nardus* on *L. monocytogenes* biofilm on stainless steel. Biofilm reduction was 44-72% on 3 and 240 h-old biofilms [45]. Number of cells of 168 h-old *L. monocytogenes* biofilm was dropped to non-detectable level after 4 hours long treatment with 0.5% thyme EO [75]. Valeriano and coworkers got the results that lemongrass EO (*Cymbopogon citratus*) had disinfection effect on 240 h-old *Salmonella enterica* biofilm on stainless steel [7]. Lemon EO had the weakest effect on bacteria thus it was not suggested to use for biofilm elimination [76, 77]. Number of survivals in one day old *Salmonella* biofilm was below 1 log CFU after 1 hour treatments with 0.05% thyme or 0.05% oregano EO [76]. One day old *E. coli* biofilm was totally eliminated with cinnamon and juniper EO [75]. Cell number of *Staphylococcus simulans, P. putida, L. monocytogenes* in 5 days-old biofilm was reduced to ≤ 2 log CFU using 20% ethanol or 8 mmol/l lactic acid or 1 mmol/l sodium hydroxide, while 1% of *Satureja thymbra* EO solved in 19% ethanol resulted full disinfection [61]. These literature data suggest that essential oils could represent alternative solution for hygiene processes against biofilms. The development of bacterial resistance to EOs is minimal [44].

7. Essential oils as food preservatives

The food industry primarily uses EOs as flavorings and their use as food preservatives requires detailed knowledge about their mode of action and the effect of the food matrix components on their antimicrobial properties [41, 42]. A range of essential oil components have been accepted by the European Commission for their use as flavorings in products (linalool, thymol, eugenol, cinnamaldehyde, vanillin, citral, limonene). These are considered to present no risk to the health of the consumer. The use of EOs as preservatives in food has been limited because high concentrations are needed to achieve sufficient antimicrobial activity. Before being added to food products, the interaction of essential oils with food components should be tested. Different strategies can be used to overcome organoleptical changes caused by essential oils. One option is to use essential oils in active packaging rather than as an ingredient in the product itself. Essential oils can be encapsulated in polymers of edible and biodegradable coatings resulting in a slow release to the food surface or to the headspace of packages of, e.g., grapes [78]. Another option would be to explore the synergism between EOs and other food preservatives. The combination of nisin with *Origanum vulgare* EO induced a synergistic effect against *L. monocytogenes* whereas the combination of nisin with *Thymus vulgaris* EO caused a synergistic effect against *Salmonella* Typhimurium [79]. Pereira de Sousa and coworkers demonstrated that sublethal concentrations of carvacrol EO and 1,8-cineol alone and in combination caused severe morphological changes in *Pseudomonas*

fluorescence that was cultivated on vegetable-broth. This indicates that the oils tested could also inhibit *P. fluorescence* growth on vegetables [80]. *Lavandula angustifolia* and *Mentha piperita* EOs caused a significant decrease of bacterial growth in minced beef stored at 9±1 °C and the addition of EOs significantly extended fresh meat odor [81]. Desai and coworkers demonstrated that by dipping catfish fillets in 2% carvacrol solution at 4°C for 30 min, reduced *L. monocytogenes* to an undetectable level from their initial load of 5 log CFU/g [82]. In celery, 1% carvacrol reduced S. *enterica* populations below the detection level just after treatment, while 1% cinnamaldehyde reduced populations by 1 and 2.3 log after treatment and 3 days later, respectively. In oysters, both antimicrobials caused about 5-log reductions after 3 days storage [83]. Regarding organoleptical changes studies show various results. The flavor of beef fillets treated with 0.8% v/w oregano oil was found to be acceptable after storage at 5 °C and cooking [84]. Oregano oil (0.05% v/w) on cod fillets produced a 'distinctive but pleasant' flavor, which decreased gradually during storage at 2 °C [85]. Labneh (Greek yogurt) containing 0.3% cinnamon, cumin or mint oils was the most acceptable and it had a good body and texture that was similar to the untreated one [86]. However, applying 1.8% of thyme oil in coating significantly decreased the acceptability of the taste of shrimps [87].

8. Conclusions

Biofilm formation of pathogenic bacteria on surfaces and on foods represents a big challenge to the food industry and healthcare. The removal of biofilms is difficult and in spite of the efforts for good sanitization surfaces and products can be contaminated which can lead to severe health problems. Quorum sensing regulates several mechanisms in bacteria, including biofilm formation. The disruption of this mechanism could represent a solution in the search for new antimicrobials. By their mode of action and composition essential oils represent an alternative to synthetic sanitizers and preservatives in the food industry against pathogenic bacteria. Biofilms investigated so far have shown higher or lower sensitivity to a number of EOs or their components *in vitro* and, also in food processing environments. Quorum sensing has also been inhibited by the EOs of several plants, herbs and spices. The organoleptic impact of essential oils and their components in food products currently limits their usage to spicy foods but several techniques like nanoencapsulation, edible coating or combination of EOs with other preservatives can represent a solution to this problem.

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